Diabetic Complications Consortium

Application Title: Human DNA methylation signatures to define diabetic cardiac subtypes

Principal Investigator: Adam R. Wende

1. Project Accomplishments:

We measured DNA methylation and gene expression in biopsy samples collected during left ventricular assist device placement. These data were analyzed three different ways: 1) diabetic versus non-diabetic, 2) ischemic versus non-ischemic, and 3) the interaction of the two. In each of these comparisons we identified significant and distinct patterns of DNA methylation. We completed this analysis with two different platforms: 1) genome-wide bisulfite sequencing (50X coverage 100 bp pair end reads) and Human Methyl450 Bead arrays. This provided us with the ability to perform cross-platform validation as well as more readily compare our results to other published studies. Our initial round of Ingenuity Pathway Analysis (IPA) and follow-up validation is underway.

Using candidate approaches from prior studies we found a number of DNA methylation sites that are differentially methylated in regions similar in both our rodent data and the current human data. Additionally, we performed RNA-sequence analysis in a subset of these samples. From those data we are working to identify which of the DNA methylation changes might contribute to functional changes in RNA abundance. Specific accomplishments are described below linked to specific aims.

Also relevant to the goals outlined in the original application, we performed this analysis on samples collected from two different sites: University of Alabama at Birmingham (n = 12) and University of Utah (n = 6). When we performed a cluster analysis, samples between the two locations grouped according to patient type (e.g. ischemic diabetic, etc.) instead of by collection site. We are working to obtain additional funding to analyze more samples to verify this finding. Finally, we have recruited an MD/PhD student (Mark Pepin, MS) to the laboratory who is currently training with the bioinformatics collaborator (Dr. Crossman) listed on this application. The first manuscript of the data will be submitted in 2016. We have presented the preliminary findings at both a local seminar and international symposium (see Section 3 below).

2. Specific Aims:

Specific Aim 1: **To determine the DNA methylation signature by which diabetes and heart failure modifies DNA to regulate gene expression.** Hypothesis – cardiac DNA is modified in response to diabetes and heart failure. To interrogate systems changes in gene expression associated with altered epigenetic modifications we will perform RNA-sequencing and bsDNA-seq on human cardiac biopsy samples with a focus on defining differences between diabetic and non-diabetic patients to identify subtypes of end-organ damage.

Results: Using RNA-sequencing we identified a set of 281 transcripts significantly regulated between diabetic and non-diabetic (n = 6). Using WebGestalt to perform Disease Association Analysis the top 5 significantly regulated pathways and genes are as listed in Table 1.

Table 1. Pathway analysis of human RNA-sequencing results. Comparison of diabetic versus non-diabetic heart failure found 281 transcripts with significant regulation. These genes were aggregated via pathway enrichment analysis for Disease Association.

Disease	#Gene	EntrezGene	Statistics
Vascular Diseases	20	8639 6347 1636 4815 1573 4878 2153 133 5320 2152 5228 12 3479 80310 4879 51330 7293 9332 4843 187	C=357;O=20;E=2.29;R=8.72; rawP=2.81e-13;adjP=2.02e-10
Cardiovascular Diseases	21	8639 2026 6347 1636 4815 1573 4878 3752 2153 133 2152 5320 5228 8048 3479 4879 80310 27063 7293 187 2702	C=425;O=21;E=2.73;R=7.69; rawP=7.89e-13;adjP=2.84e-10
Pathologic Processes	21	5999 627 4004 6347 5654 1636 63976 6275 3664 2153 27184 2214 595 10631 94103 2947 4879 51330 2944 2118 4843	C=561;O=21;E=3.60;R=5.83; rawP=1.40e-10;adjP=2.52e-08
Genetic Predisposition to Disease	25	5999 627 1394 23017 6347 5654 1636 4878 1277 51196 5142 3664 641 2153 2214 595 11322 114815 7049 1583 94103 12 2947 2944 4843	C=808;O=25;E=5.19;R=4.82; rawP=1.31e-10;adjP=2.52e-08
Stress	19	1263 8639 627 1394 1718 4170 467 641 9252 2353 26287 3725 1647 2027 58 2947 4879 2944 4843	C=464;O=19;E=2.98;R=6.38; rawP=2.44e-10;adjP=2.92e-08

This analysis was completed in July. We have subsequently sequenced an additional 12 samples, (sequencing completed in October) and we are currently combining these data sets.

Using Methyl450 Bead Array analysis (n = 12) we identified four distinct methylation patterns: non-diabetic ischemic, non-diabetic non-ischemic, diabetic ischemic, and diabetic non-ischemic as defined in Figure 1.

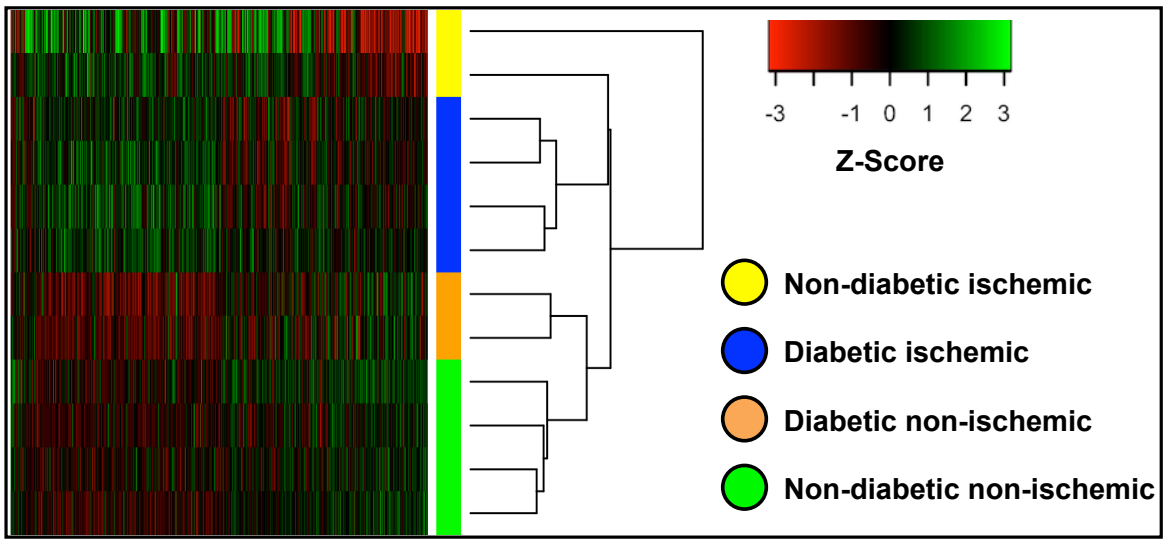

Figure 1. Hierarchical clustering of 1000 most significant CpG methylation changes. Comparison of 12 patient samples revealed four distinct patterns of DNA methylation.

We have analyzed an additional six samples by both Methyl450 Bead Array and genome-wide bisulfite sequencing. That data collection was completed earlier this month and we are currently combining these different data sets for further analysis. Initial principle components analysis has the samples grouping by patient type (four groups listed above). Together this data supports that there is a significant and robust change in DNA methylation related to both the diabetes status of the patient as well as their heart failure etiology. All data sets will be uploaded to GEO following analysis and made public following publication.

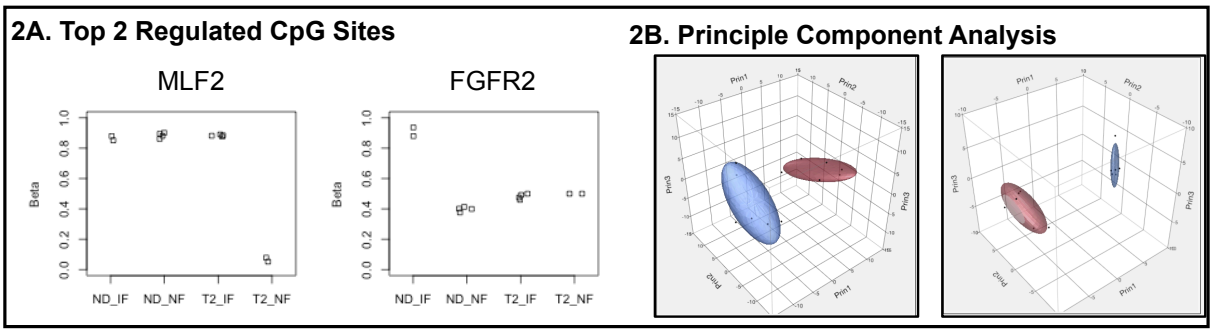

Figure 2. Top two CpG sites and PCA analysis of DNA methylation changes. A) Example of top 2 most significantly regulated CpG sites, and listed nearest gene. ND_IF = non-diabetic ischemic failure; ND_NF = non-diabetic non-ischemic failure; T2_IF = diabetic ischemic failure; and T2_NF = diabetic non-ischemic heart failure B) PCA clustering between ischemic and non-ischemic sample (left) or diabetic and non-diabetic (right).

To begin to define potential roles for this regulation we subjected each of the lists generated for significant changes to IPA analysis as described above. The top network regulated in diabetic versus non-diabetic is shown in Figure 3.

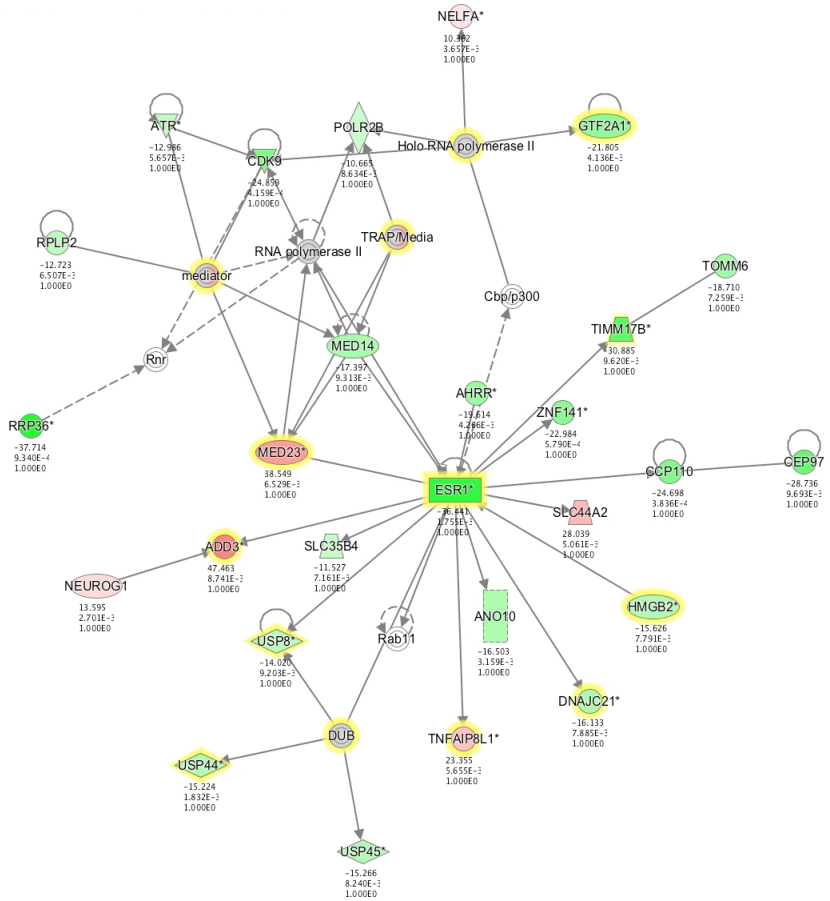

Figure 3. Top Network identified by Ingenuity Pathway Analysis in Diabetic Samples. Using a 10% differential methylation cutoff, a $P < 0.01$, and only including CpG sites in known CpG islands we identified 396 significant changes in non-diabetic versus diabetic samples, independent of heart failure etiology. The top Network is shown here.

Specific Aim 2: **To determine the conservation of DNA changes in diabetes and heart failure between rodent models and humans and test functional relevance.** Hypothesis – determining evolutionarily conserved molecular mechanisms of gene regulation is required to establish models of human disease. Building on current studies in the laboratory and public databases, we will use both candidate gene and bioinformatic approaches to identify changes in DNA modifications that are conserved in mouse models of diabetic cardiac complications and human disease associated with altered gene expression.

Results:

We have compared the differentially methylated sites identified in our diabetic and an inducible glucose transporter 4 (GLUT4)-overexpression mouse model with sites identified in the published literature and our human data generated in Aim 1. Top targets include BDH1, CPT1A, COX6A2 and many others. For example in diabetic patients versus non-diabetic BDH1 methylation increased 2.97-fold, similar to that observed in mouse diabetic heart tissue. In both our mouse and cell culture studies we have already begun to characterize *Bdh1* promoter methylation which is associated with a marked decrease in transcript levels as identified by either microarray or RNA-sequencing, Figure 4. We are in the process of performing additional cross-species comparisons and validations. A post-doctoral fellow in the laboratory (Dr. Manoja Brahma) is comparing this regulation in our mouse models.

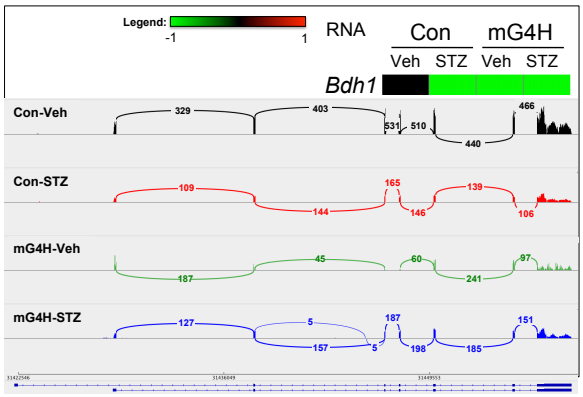

Figure 4. Example gene target identified by comparing differentially methylated genes in both human and mouse diabetic heart samples. Microarray analysis (n = 6) of mouse heart RNA in 4 weeks diabetic (STZ) with or without 2 weeks of cardiomyocyte GLUT4 transgene induction (mG4H) (top) and RNA-sequence (n = 3) analysis in the same four groups visualized by alignment using Integrative Genomics Viewer (Broad).

3. Publications and Presentations:

We have summarized the interplay of posttranslational regulation and gene expression in connection with DNA methylation or other diabetic complications. This is highlighted in a review and commentary. Of note we have also disseminated the preliminary human data described above at both local and international meetings. One of these abstracts was selected for a short talk and another was an invited seminar. We expect to submit manuscript(s) describing the human results from this project in 2016.

1. **Wende AR**. Post-translational modifications of the cardiac proteome in diabetes and heart failure. Proteomics Clinical Applications, 2015. [Epub ahead of print] PMID: 26140508

2. **Wende AR**. Unsticking the broken diabetic heart: *O*-GlcNAcylation and calcium sensitivity. Diabetes, 64(10):3339-3341, 2015. PMID: 26405271 4.
3. Crossman DK, Brahma MK, McCrory MA, Drakos S and **Wende AR**. Post-translational regulation of cardiac gene expression and glucose-mediated regulation of DNA methylation. SHVM – Protein and amino acid metabolism and the role of post-translational protein modifications on substrate metabolism: Cardiovascular consequences, 2015. (poster and selected talk)
4. **Wende AR**. Approaches to bridge the gap between basic and clinical epigenetics research. Epidemiology Seminar Series, UAB, 2015. (invited speaker)
5. Pepin ME, Crossman DK, Brahma MK, McCrory MA, Barchue JP, Pogwizd SM, Drakos S and **Wende AR**. Human DNA methylation signatures to define diabetic cardiac subtypes. [in preparation]
6. Brahma MK, McCrory MA, Paterson AJ, Pepin ME, Young ME and **Wende AR**. Regulation of myocardial ketone oxidative proteins by increased *O*-GlcNAcylation. [in preparation]